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Antioxidant and hypoglycaemic effects of local bitter gourd fruit (*Momordica charantia*)

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Abstract: Antioxidant and anti diabetic properties of two local bitter gourd species namely *peria kambas* and *peria katak* were screened with 3 antioxidant assays (2,2-diphenyl-1-picrylhydrazyl [DPPH], the ability of ferric ion reduction in plasma [FRAP] and total phenolic content [TPC]) and 2 inhibition assays of key enzymes in carbohydrate metabolism (α-amylase and α-glucosidase). Mature *peria katak* appeared as stronger antioxidant vegetables in three antioxidant assays (51.1 % in DPPH inhibition, 0.63 g gallic acid equivalent and 2.29 g Trolox equivalent/100 g dried weight of peria katak) than *peria kambas*. Besides, it also reported to be 21% and almost three fold stronger in inhibiting the activity of enzymes α-amylase and α-glucosidase as compared to another bitter gourd cultivar. These pharmacology properties of *peria katak* were further determined along the ripening stages. Again, it was found that mature peria katak showed the highest antioxidant potential as well as the highest half maximal inhibitory concentration (IC_{50}) values of 0.63 mg/mL and 0.62 mg/mL for α-amylase and α-glucosidase enzyme inhibition assays respectively. Interestingly, mature peria katak was more effective than acarbose, one of the commonly used oral anti diabetic drug in inhibiting α-amylase activity and almost as good as acarbose in inhibiting α-glucosidase activity. In conclusion, peria katak is more effective than peria kambas in suppressing free radical and decreasing hyperglycemia post-ingestion. Therefore, the local mature peria katak can serve as a better antioxidant and anti diabetic tool in food and nutraceutical product development.
Keyword: bitter gourd, ripening stage, antioxidant, anti diabetic, acarbose.

Introduction

Type 2 diabetes mellitus occurs when the pancreas does not produce sufficient insulin, or when the body cannot effectively use the insulin it produces to monitor the glucose uptake by body cells and eventually lead to hyperglycemia. The study of Wan Nazaimoon et al.[1] recorded an overall diabetes prevalence of 22.6%, almost a two-fold increase from 11.6% reported in 2006. Shaw et al.[2] also predicted that Malaysia will be the tenth country of highest prevalence of diabetes mellitus. This alarming increase in the prevalence of diabetes in Malaysia is mainly due to population growth, obesity, ageing, unhealthy dietary intake and lack of exercise. Currently, type 2 diabetes mellitus is controlled and managed by a combination of diet restriction, weight reduction through regular exercise and oral hypoglycaemic drugs such as metformin, acarbose and others. When hyperglycemia becomes severe, patients are usually switched to insulin injections, with or without oral agents to improve insulin action.

Oxidative stress has been strongly associated with the occurrence of type 2 diabetes. This is because the insulin deficiency among diabetic patients may promote β-oxidation of fatty acids and results in the generation of hydrogen peroxide[3]. The production of reactive oxygen species (ROS) such as hydrogen peroxide will in turn

reduce the insulin secretion by liver and glucose utilization by peripheral tissues. However, conventional anti-diabetic therapy mainly focuses in maintaining blood glucose level in blood but not oxidative damage. In addition, it also frequently related to the side effect of oral agents, does not effectively maintaining euglycemia level and causes serious complications under long term consumption. Therefore, some local patients tend to go for alternative treatment for instance herbal medicine, Chinese traditional medicine and ayuverdic medicine which treat both illnesses in the same time with lower cost.

Over 1000 plants had been recognized to possess antioxidant and anti-diabetic potential including *Momordica charantia*. It belongs to family Cucurbitaceae and commonly known as bitter gourd or *peria* in Malaysia. Local species of bitter gourd consists of 2 types: (i) *peria kambas* (long light green fruit) and (ii) *peria katak* (small dark green fruit). Bitter gourd has received growing attention among all vegetables crops nowadays because it contains an abundance of phytochemicals that associated with antioxidants, anti diabetes, anti microbial, anti cancer, hypertensive properties and others[4]. To date, little is known about the changes in pharmacology of local bitter gourd affected by species and ripening stages. Thus, the objective of this study was to evaluate the influences of species and ripening stages (raw, mature and overripe) on the *in vitro* antioxidant and hypoglycaemic effects of local bitter gourd fruits.

Materials and Methods

Materials

Apparently fresh mature *peria kambas* (LBG) and *peria katak* (SBG) were purchased from local market, Kajang while fresh SBG of different ripening stages were harvested from Lekir Agricultural Department, Perak. SBG were collected according to three ripening stages: (i) raw, (ii) mature and (iii) ripe. Raw SBG were harvested when the fruits look green with or without immature seed (about 2 weeks from flowering); mature SBG were harvested when the fruits look green, less firm and with mature seed (about 3 weeks from flowering); overripe SBG were harvested when the fruits look yellow with red seeds (about 4 weeks from flowering). All fruits were washed cleanly, chopped into small pieces and dried in oven (Beschickung-Loading Modell 100-800, Memmert, Germany) at the temperature of 50°C for 32 hours until the weight reached unchangeable. Dried bitter gourd fruits were grinded into powder and kept in air tight container at chilling temperature.

Extraction of phytochemical

Fruit powder (50 g) was extracted by ethanol 50% with hot reflux extractor at the temperature of 150°C for 6 hours. Extracts were then concentrated and dried using rotary vacuum evaporator prior using in the following analysis.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging system

The determination of antioxidant activity through the DPPH scavenging system was carried out according to the method of Musa et al.[5]. Methanolic DPPH solution with the absorbance of 1.00±0.01 unit at 516 nm wavelength was prepared freshly before use. One hundred microliter of bitter gourd fruit extracts (50 mg/mL 50% ethanol) with 1.5 mL DPPH solution prepared were kept overnight for scavenging reaction in the dark. An aliquot (200 μL) of samples (bitter gourd fruit extracts with methanolic DPPH solution) and blank were then monitored at 516 nm wavelength on the next day with the spectrophotometer (Epoch, Biotek, USA). Percentage of DPPH scavenging activity was determined as follow:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{absorbance of blank-absorbance of sample})}{\text{absorbance of blank}} \times 100\%$$

Ferric reducing ability on plasma (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of Musa et al.[5]. FRAP reagent was prepared fresh as using 300 mM acetate buffer (pH 3.6); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCl; and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 0.5 mL FRAP reagent was added to 50 μL bitter gourd fruit extracts (50 mg/mL 50% ethanol) and the absorbances were taken at 595 nm wavelength with the spectrophotometer after 30 minutes. The calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dried sample (mg TE/100 g of DW).

Total phenolic content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of Musa et al.[5]. About 50 µL bitter gourd fruit extracts (50 mg/mL in 50% ethanol) were added with 0.2 mL distilled water and 0.25 mL diluted Folin-Ciocalteu reagent. The samples (bitter gourd fruit extracts with Folin-Ciocalteu reagent) were left for 5 minutes before 0.5 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with the spectrophotometer after 2 hours. The calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dried sample (mg GAE/100 g of DW).

α-amylase enzyme inhibition

The activity of α-amylase enzyme inhibition was determined through iodine-starch test[6]. The total volume of sample consisted of 120 uL 0.1M sodium phosphate buffer, 40 uL α-amylase (1 U/mL) and 100 uL extract (0.2-1.0 mg/mL). The mixture was incubated for 15 minutes at 37°C. About 100 uL soluble starch (0.2%) was added to the sample and the mixture was reincubated. The enzyme activity was stopped by the addition of 40 uL 1 M hydrochloric acid and hot water bath (5 minutes). Iodine reagent (100 uL) was lastly added to the mixture before the absorbances were taken at 620 nm wavelength with the spectrophotometer. Acarbose and distilled water were used as positive and negative control in the test. Percentage of α-amylase enzyme activity was determined as follow:

$$\text{Relative } \alpha\text{-amylase enzyme activity (\%)} = \frac{\text{enzyme activity of sample}}{\text{enzyme activity of negative control}} \times 100\%$$

α-amylase enzyme inhibition (%) = 100% – Relative α-amilase enzyme activity (%)

The IC_{50} values were determined by the calibration curve of α-amylase enzyme inhibition against concentration of extracts.

α-glucosidase enzyme inhibition

The activity of α-glucosidase enzyme inhibition was determined using p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate[7]. About 650 uL phosphate buffer (67 mM, pH 6.9) and 100 uL extract (0.2-1.0 mg/mL) were mixed with 100 uL α-glucosidase (0.5 U/mL). The mixture was incubated for 15 minutes at 37°C. Two hundred and fifty microliter of pNPG (10mM in phosphate buffer) was then added to the sample and the mixture was reincubated. The enzyme activity was stopped by the addition of 250 uL 0.1 M sodium carbonate. Enzyme activity was measured by the absorbance of liberated p-nitrophenol from pNPG at the wavelength of 405 nm using spectrophotometer. Acarbose and distilled water were used as positive and negative control in the test. Percentage of α-glucosidase enzyme activity was determined as follow:

$$\alpha\text{-glucosidase enzyme inhibition (\%)} = \frac{\text{(absorbance of negative control-absorbance of sample)}}{\text{absorbance of negative control}} \times 100\%$$

The IC_{50} values were determined by the calibration curve of α-glucosidase enzyme inhibition against concentration of extracts.

Statistical analysis

The *in vitro* antioxidant and anti-diabetic properties between two local species of bitter gourd (LBG and SBG) were firstly investigated. The effect of ripening stages was then studied using the species of bitter gourd with stronger antioxidant and hypoglyceamic properties. All the data collected was analyzed statistically using Statistical Packages for the Social Sciences (SPSS) 20.0. Differences among species and ripening stages were analyzed respectively by t-test and Tukey's method. Significant level used was $p \leq 0.05$ for all data analyzed.

Results and Discussion

Antioxidant properties of bitter gourd

Table 1 showed the antioxidant properties of local bitter gourd. Mature SBG had stronger antioxidant capacity out of all assays run as compared to mature LBG ($p \leq 0.05$). The different capacity of antioxidant in both species was most probably due to the variation of hydrophilic phytochemical extracted from the fruit such as vitamin C, vanilic acid, gallic acid, tannic acid, catechin, p-coumaric acid, benzoic acid and others. On the other hand, mature SBG consistently reported to exhibit the strongest antixidant capacity in DPPH, FRAP and TPC assays, followed by raw and overripe SBG (Table 2). The antioxidant properties of mature SBG were about 1.5-2.3-fold stronger than that of the raw and overripe SBG.

Table1. Antioxidant and anti-diabetic properties of local bitter gourd (Momordica charantia)

Pharmacology properties		*Peria kambas*	*Peria katak*
Antioxidant	DPPH scavenging system (%)	33.7^{b}	51.1^{a}
	FRAP (g TE/100 g dried bitter gourd)	0.45^{b}	0.63^{a}
	TPC (g GAE/100 g dried bitter gourd)	1.81^{b}	2.29^{a}
Anti-diabetic	IC_{50} of α-amylase acitivity inhibition (mg/mL)	0.76^{b}	0.63^{a}
	IC_{50} of α-glucosidase acitivity inhibition (mg/mL)	1.68^{b}	0.62^{a}

n=3.
Different superscripts letter indicates significant statistical difference in row ($p \leq 0.05$).
DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: ferric reducing ability on plasma; TE: Trolox equivalent; TPC: total phenolic content; GAE: gallic acid equivalent.

Table 2. Effect of ripening stage on *peria katak*'s antioxidant properties

Ripening stage	DPPH scavenging system (%)	FRAP (g TE/100 g dried bitter gourd)	TPC (g TE/100 g dried bitter gourd)
Raw	33.0^{b}	0.38^{b}	1.53^{b}
Mature	51.1^{a}	0.63^{a}	2.29^{a}
Overripe	27.6^{c}	0.27^{c}	0.96^{c}

n=3.
Different superscripts letter indicates significant statistical difference in column ($p \leq 0.05$).
DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: ferric reducing ability on plasma; TE: Trolox equivalent; TPC: total phenolic content; GAE: gallic acid equivalent.

Bitter gourd may inhibit the generation of ROS through both enzymatic and non-enzymatic pathway[8]. In enzymatic pathway, antioxidant enzymes such as superoxide dismutase destroys the superoxide radicals this produces hydrogen peroxide which is harmful to human body while catalase and peroxidase functions in detoxification of hydrogen peroxide into water and hydrogen. For non-enzymatic antioxidant pathway, bioactive compounds in bitter gourd contribute in removing or inactivating the free radical thus minimizing the oxidative stress-induced damages. Several compounds in bitter gourd have been identified to be related to the their antioxidant activities including phenolic acids (gallic acid, salicylic acid, cinnamic acid, chlorogenic acid, ferullic acid and so on), myricetin, quercetin, ascorbic acid and triterpenoids[9].

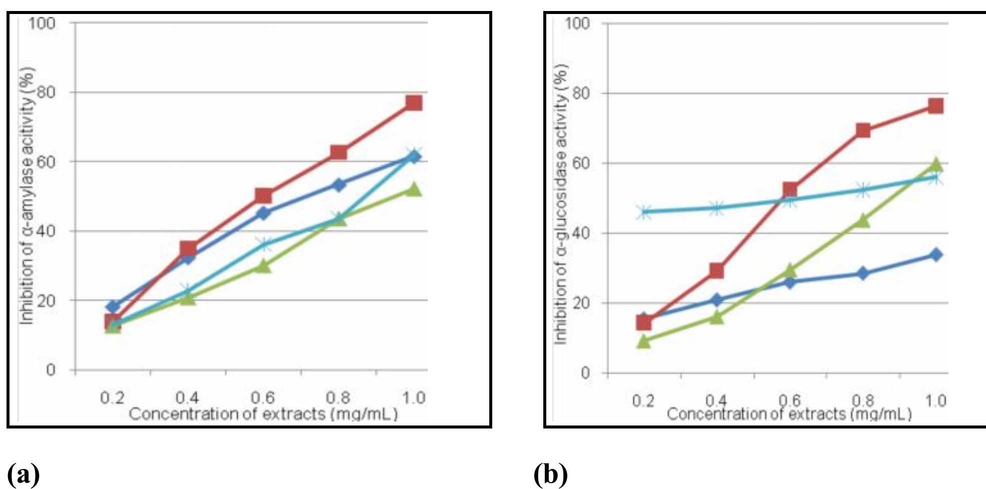
Bitter gourd fruit has known to be a richer source of antioxidant compounds than some common vegetables such as cabbage, celery, eggplant, onion, green pepper, snap bean, tomato, radish, wild carrot, winter melon and ribbed gourd[10]. Furthermore, Wu and Ng[11] had concluded that bitter gourd fruits exhibited better radical scavenging effect and metal chelating ability than vitamin E in their study. Islam et al.[12] reported higher scavenging of free radical in other species of bitter gourd including Indian Green bitter gourd, Indian White bitter gourd, China Green bitter gourd and China white bitter gourd (79-87%) but lower TPC content among the species (4.6-8.0 mg/g dry matter). Lu et al.[13] also agreed with the strong but big variation of antioxidant and anti-radical activities of 16 wild bitter gourd cultivars in Taiwan.

However, the effect of ripening stages on antioxidant activity of bitter gourd was inconclusive. Ozysaglam and Karakoca[14] summarized that the ripe bitter gourd fruit extract exhibited the strongest antioxidant activities compared with unripe fruit via DPPH (45.95%), FRAP, TPC (23.45 mg gallic acid equivalent/g dry extract), total antioxidant capacity (81.46 mg ascorbic acid equivalent/g dry extract) and cupric

ion reducing antioxidant capacity assays while Kubola and Siriamornpun[15] reported that green bitter gourd fruit (32.4 mg gallic acid equivalent/100g fresh weight) had higher values of TPC than the ripe fruit (22.4 mg gallic acid equivalent/100g fresh weight). Anyway, the study of Aminah and Anna[16] showed that there was no significant different for the values of DPPH and FRAP of young, mature I, mature II dan overripe bitter gourds.

Hypoglyceamic properties of bitter gourd

SBG found to possess a more favourable α-amylase and α-glucosidase inhibitory effect on starch breakdown than LBG (Table 1). This was parallel with the local folk medicine that SBG is effective in handling diabetes. Out of 3 ripening stages of SBG investigated, the mature SBG strongly inhibited the activity of α-amylase, followed by raw and overripe SBG while the mature SBG, again, strongly inhibited the activity of α-glucosidase, followed by overripe and raw SBG (Figure 1). One of the popular oral antidiabetic drug, acarbose was the positive control used in both assays. Surprisingly, mature SBG showed better α-amylase inhibition activity than anti-diabetic drug, acarbose (IC_{50} for mature SBG: 0.63 mg/mL; IC_{50} for acarbose: 0.86 mg/mL) and comparable to acarbose in inhibiting α-glucosidase activity (Table 3).



(a) (b)

Figure 1. Effect of ripening stage on *peria katak*'s anti-diabetic properties (a) Inhibition of α-amylase activity (b) Inhibition of α-glucosidase activity. (♦: raw bitter gourd; ■: mature bitter gourd; ▲: overripe bitter gourd; ж: oral anti-diabetic drug, acarbose)

Table 3. Effect of ripening stage on *peria katak*'s anti-diabetic properties

Samples	Raw fruit	Mature fruit	Overripe fruit	Acarbose
α-amylase activity inhibition (IC_{50}, mg/mL)	0.75^b	0.63^a	0.96^d	0.86^c
α-glucosidase activity inhibition (IC_{50}, mg/mL)	1.73^d	0.62^b	0.88^c	0.59^a

n=3.
Different superscripts letter indicates significant statistical difference in row (p≤0.05).

α-amylase is involved in the breakdown of long chain carbohydrate and α-glucosidase breaks down starch and disaccharides to glucose. Inhibition of these enzymes helps to reduce the rate of carbohydrate digestion, delay the intestinal absorption and slow down the sharp rise in blood sugar levels that diabetic patients typically experience after meals[17]. In α-amylase inhibition assay, the breakdown of starch was minimized, yielding dark blue colour of solution after the addition of iodine in control and samples. In contrast, enzyme inhibitor decelerates the breakdown of pNPG by α-glucosidase, producing the yellow colour solution due to the liberation of p-nitrophenol from pNPG.

Inhibition of these two enzymes lowers the content of sugar available for absorption during digestion thus it can be used as a good strategy in the early treatment of type 2 diabetes mellitus. In this study, LBG and young SBG were found to be more effective in inhibiting the activity of α-amylase while mature and overripe SBG were almost equally capable in inhibiting activities of both enzymes. Although the reported values of enzymes inhibition were higher than that of the previous studies, the inhibition trend was very much different from each other. Wongsa et al.[18] revealed that bitter gourd has stronger inhibition ability in α-glucosidase (>30%) than α-amylase (<15%). Ahmad et al.[19] also made similar conclusion where oil extracted from bitter

gourd was able to reduce 25-38% of α-amylase activity and 20-54% of α-glucosidase activity while polypeptide k from bitter gourd reduce 18-36% of α-amylase activity and 45-79% of α-glucosidase activity respectively.

Enzyme inhibitor such as acarbose aid in minimizing the activity of amylase and glucosidase at the brush boarder of small intestine, restricting the breakdown of starch and sucrose, delaying the digestion complex carbohydrate and absorption of glucose in alimentary tract, finally slowing down the rise of glucose in blood after meal[20,21]. The result of study suggested that SBG could serve as a potent carbohydrate-hydrolyzing enzyme inhibitor because it was as effective as acarbose in its action. Furthermore, the substitution of acarbose by SBG may avoid its side effects of gastrointestinal discomfort[22]. Other than this, bitter gourd is also believed to exert other hypoglycaemic effects such as insulin secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of hexokinase activity and preservation of pancreatic islet cells and their functions[23].

Conclusion

Local mature SBG showed the greatest ability in suppressing free radical and decreases hyperglycaemia post-ingestion through the inhibition of key enzymes in carbohydrate metabolism. It is therefore suggested that it can serve as a potent antioxidant and anti-diabetic tool in food as well as nutraceutical product development.

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